Ocular Lesions in Canine Mucopolysaccharidosis I and Response to Enzyme Replacement Therapy

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PURPOSE. Mucopolysaccharidosis I (MPS I) is an inherited metabolic disorder resulting from deficiency of α-L-iduronidase and lysosomal accumulation of glycosaminoglycans (GAG) in multiple tissues. Accumulation of GAG in corneal stromal cells causes corneal opacity and reduced vision. The purpose of this study was to determine the extent of ocular GAG accumulation and investigate the effectiveness of intravenous enzyme replacement therapy (ERT) on corneal GAG accumulation in dogs.

METHODS. Ocular tissues were obtained from 58 dogs with mucopolysaccharidosis I and four unaffected controls. Affected dogs received either low-dose ERT, high-dose ERT, or no treatment; some low-dose dogs also received intrathecal treatments. Histologic severity of corneal stromal GAG accumulation was scored.

RESULTS. Accumulation of GAG was found in corneal stromal cells and scleral fibroblasts but not in corneal epithelium, endothelium, ciliary epithelium, choroid, retina, retinal pigment epithelium, or optic nerve. Corneal GAG accumulation increased in severity with increasing age. Although low-dose ERT did not significantly reduce corneal stromal GAG accumulation in comparison with untreated animals, high-dose ERT did result in significantly less GAG accumulation compared with the untreated dogs (adjusted $P = 0.0143$) or the low-dose ERT group (adjusted $P = 0.0031$). Intrathecal treatments did not significantly affect GAG accumulation. Dogs that began ERT shortly after birth also had significantly less ($P < 0.0001$) GAG accumulation in the corneal stroma than dogs with a later onset of treatment.

CONCLUSIONS. These data suggest that high-dose, intravenous ERT is effective at preventing and/or clearing corneal stromal GAG accumulation, particularly if initiated early after birth. (Invest Ophthalmol Vis Sci. 2011;52:5130–5135) DOI:10.1167/iovs.10-6751

Mucopolysaccharidosis I (MPS I) is due to a deficiency of the enzyme α-L-iduronidase, which hydrolyzes the terminal α-iduronic acid of dermatan and heparan sulfates. This defect results in multisystemic accumulation of partially catabolized glycosaminoglycans (GAG) in lysosomes of fibroblasts, as well as fixed macrophages, chondrocytes, myocytes, and pericytes. The condition is autosomal recessive and is further characterized as Hurler, Scheie, or Hurler-Scheie syndrome based on the severity of disease. Those with Hurler syndrome (MPS IH) are most severely affected, while patients with Scheie syndrome (MPS IS) are the least affected. Hurler syndrome in humans is associated with marked corneal opacification, facial and skeletal dysmorphism, behavioral or intellectual impairments, cardiac and respiratory disease, hepatosplenomegaly, and a shortened lifespan. Patients with Scheie syndrome have mild corneal opacity, characteristic facial changes and musculoskeletal problems, normal intelligence, milder cardiac and respiratory disease, and hepatosplenomegaly but have a normal lifespan.

MPS I as reported in cats and in Plott hound dogs most closely resembles the intermediate Hurler-Scheie (MPS IHS) form of the disease. Affected dogs are stunted and develop progressive lameness due to degenerative joint disease and consistently develop corneal opacities by 1 year of age.

Normal corneal transparency is attributed to a lack of vascularization, non-keratinized surface epithelium, a lack of pigment, and to the size and organization of stromal collagen fibrils. Collagen fibrils, keratan sulfate, heparan sulfate, and glycoproteins compose 15% to 25% of the corneal stroma, with water making up the vast majority of the remaining stroma. The proteoglycans in the cornea are involved in collagen fibril ultrastructure, packing, organization, and size, as well as stability of the corneal lamellae. Keratan sulfate and dermatan sulfate, made by corneal stromal cells (keratocytes) and corneal epithelial cells, respectively, are the most abundant stromal GAG. Any change in GAG content can affect the function of Na+/K+ ATPase pumps in the corneal epithelium and corneal endothelial cells and result in corneal edema and opacity. In humans with MPS I, corneal opacification is attributed to intracellular and extracellular GAG accumulation in keratocytes, stroma, and endothelial cells. Reports vary regarding the accumulation of GAG in human corneal epithelial cells. In dogs with MPS I, GAG accumulation in keratocytes is correlated with a sixfold increase in corneal dermatan sulfate. Furthermore, separation of corneal stromal collagen fibrils by > 2000 angstroms results in increased diffraction of light and subsequent corneal clouding. Accumulation of GAG within keratocytes in cats, dogs, and humans is believed to result in changes in collagen fibril diameter and spacing sufficient to result in corneal opacity.

Several treatment modalities for MPS I have been investigated in animal models and humans. Bone marrow transplantation (BMT) has been used to deliver a self-renewing population of cells capable of producing the deficient enzyme, and this alleviates some symptoms, including corneal opacity, in dogs. In another canine study, BMT decreased systemic GAG accumulation, but corneal GAG accumulation and clouding persisted to some degree. In humans, BMT effectively reduces visceral GAG accumulation; efficacy in treating the eye, central
nervous system, and musculoskeletal system is less clear.\textsuperscript{12} Given the high morbidity and mortality associated with BMT, other treatment modalities have been developed and investigated.

Intravenous (IV) enzyme replacement therapy (ERT) with recombinant human α-L-iduronidase ameliorates some symptoms in human patients with MPS I.\textsuperscript{13} In humans, IV ERT decreases systemic GAG accumulations; however, no significant improvement in corneal opacity has been reported.\textsuperscript{14,15} In a low-dose, long-term canine study (25,000 units/kg IV weekly for 13 months; equivalent to approximately 0.1 mg/kg), the highest levels of recombinant human enzyme activity were in the liver, with lower levels in the spleen, kidney, brain and heart; no enzyme activity was detectable in cartilage or in the cornea.\textsuperscript{16} In that study, no clinical improvement in corneal opacity was noted after treatment. In another study, five high-dose treatments (125,000 units/kg; equivalent to approximately 0.5 mg/kg) over the course of 10 days did result in detectable corneal enzyme activity, but levels were still lower than in control animals.\textsuperscript{16} In a related study, no detectable corneal enzyme activity was found in three affected dogs after weekly treatments with 250,000 units/kg (1 mg) (equivalent to approximately 19,000 units/kg or 0.08 mg/kg), and there was no change in keratoocyte GAG accumulation.\textsuperscript{17} A more recent study noted some clinical and histologic improvement of corneal lesions with high-dose IV ERT (1.57 mg/kg weekly).\textsuperscript{18}

We investigated histologic GAG accumulation in ocular tissues archived from MPS I-affected dogs that had received various doses and regimens of ERT to determine the effects of these treatments on reducing corneal GAG accumulation. This is the first large-scale report of corneal response to treatment after IV ERT.

\section*{Methods}

\subsection*{Animal Information}

This retrospective study evaluated ocular tissues previously collected from animals used in various therapeutic experiments. Animals were from a colony of Plott hound-beagle crosses originally established at the University of Tennessee in 1982\textsuperscript{3} and subsequently moved to the University of California, Los Angeles. Affected dogs in this colony are homozygous for a null mutation in α-L-iduronidase.\textsuperscript{7} Dogs received weekly IV ERT as previously described.\textsuperscript{16} Animals in the low-dose groups received 0.58 mg/kg of α-L-iduronidase, whereas the high-dose group received 1.58 to 2 mg/kg. In addition to the IV ERT, 20 of the low-dose dogs also received at least one intrathecal (IT) treatment; in most cases the intrathecal treatments were performed monthly for 3 to 6 months. In both the low- and high-dose groups, some dogs (8 of 35 and 8 of 14, respectively) began ERT within a month of birth, while treatment in other dogs did not begin until at least 6 months of age. After euthanatization, ocular tissues were removed from 58 MPS I-affected and four unaffected dogs that were part of these studies. All therapeutic studies were preapproved by the Institutional Animal Care and Use Committee and adhere to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research; further details concerning these animals have been reported elsewhere.\textsuperscript{17–22}

\subsection*{Microscopic Evaluation}

After euthanatization, ocular tissues were removed and fixed in 10% neutral buffered formalin and routinely processed for histologic examination. In 57 of 62 dogs (92%), the entire globe was available for examination; remaining samples consisted of only cornea. The severity of corneal GAG accumulation was graded histologically by a blinded board-certified pathologist (KMN) on a scale of 0 to 4, using hematoxylin and eosin (HE)-stained sections. Lesions graded 0 had no detectable GAG accumulation (cytoplasmic vacuolation). Lesions graded 1 had infrequent corneal stromal cells with detectable GAG. Grade 2 lesions had multifocal or focally extensive areas of GAG accumulation. Grade 3 lesions also had diffuse, mild GAG accumulations, which did not separate collagen fibers. Lesions graded 4 had diffuse GAG accumulation, which was sufficient to separate collagen fibers, and aggregates of foamy cells. Generally both eyes were equally affected; if one cornea was more severely affected, it was used for scoring. Histochemistry (Alcian blue) was performed on some slides. In addition, immunohistochemistry for CD18 (mouse monoclonal, clone Ca16.3c10; provided by Peter F. Moore, University of California, Davis) was performed on some sections to detect histiocytes. Briefly, slides were deparaffinized, dehydrated, and treated with proteinase K (Dako, Carpinteria, CA) for antigen retrieval. Sections were then blocked with 3% hydrogen peroxide and incubated with a 1:10 dilution of anti-CD18 antibody for 30 minutes, followed by a 30-minute incubation with the secondary antibody (EnVision+ System HRP Anti-Mouse; Dako) and a 10-minute detection step using DAB+ (Dako). Slides were counterstained with hematoxylin (Hematoxylin 2; Richard Allen Scientific, Kalamazoo, MI), dehydrated, and cover slipped.

Corneal samples from two affected dogs in the low-dose group with grade 4 GAG accumulation were transferred from formalin to Carson’s fixative for ultrastructural examination. Samples were washed in phosphate buffer, postfixed for 60 minutes in buffered 2% osmium tetroxide, rinsed, and dehydrated in a graded ethanol series before embedding in Spurr epoxy. Thin sections were cut on a microtome (OMU3 Ultramicrotome; Reichert, Depew, NY), stained with uranyl acetate and lead citrate, and then examined with an electron microscope (Hitachi H800; Hitachi, Tokyo, Japan). Images were recorded on electron microscope film (Kodak 4489; Kodak, Rochester, NY).

\subsection*{Statistical Analysis}

Unaffected control MPS I carriers were excluded from all statistical analyses. The effects of age (months) and treatment group on histologic grade were evaluated using a mixed model ANOVA procedure with histologic grade included as an ordinal dependent variable. The model to evaluate the effect of dose (high versus low) on duration of treatment included treatment at birth and the interaction of treatment at birth and dose as independent variables. A third model to evaluate the effect of treatment at birth on histologic grade was evaluated using a mixed model ANOVA and included treatment at birth, dose (high versus low), duration of treatment, age, and the interaction between duration of treatment and treatment at birth, and dose and treatment at birth as independent variables in the model. The Pearson correlation coefficient was used to assess the association of histologic grade with duration of treatment. To assess the effect of intrathecal treatment on histologic grade, treatment at birth, age, and duration of treatment were included as covariates in the model. Data were transformed as necessary to meet the assumption that the residuals from the models fit a normal distribution. The fit of the residuals from the models to a normal distribution was evaluated with the test statistic of Shapiro-Wilk.

\section*{Results}

\subsection*{Animal Information}

Corneas from 62 dogs were examined. These included four unaffected, control dogs; nine affected, untreated dogs; 35 affected dogs in the low-dose group, and 14 affected dogs in the high-dose group. Untreated, affected dogs had distinct opacification of the cornea (Fig. 1), but detailed clinical examination was not performed on most dogs in these studies. Most samples consisted of the entire globe; only cornea was available from two unaffected, control dogs; two affected, low-dose dogs; and one affected but untreated dog.

\subsection*{Microscopic Evaluation}

In affected eyes, corneal stromal cells were variably distended by foamy cytoplasm. The cytoplasmic vacuolation tended to be...
more severe in the deeper layers of the corneal stroma. Examples of the histology scores are shown in Figure 2. The distribution of ages and severity of corneal GAG accumulation by treatment group are shown in Tables 1 and 2. In almost all cases, there was concurrent corneal vascularization. Some dogs had mineralization of the corneal epithelial basement membrane. Uncommonly, the vacuolated cells coalesced, resulting in marked separation of fibers and cleft formation. Rarely, areas of stromal necrosis (loss of keratocytes) were present.

This storage material stained blue-green with Alcian blue (Fig. 2F). On ultrastructural examination stromal cells were expanded by lysosomes containing flocculent granular material (Fig. 3). In some cases there was increased cellularity in the most severely affected areas of the cornea: immunohistochemistry for CD18, a marker for canine histiocytes, highlighted increased immunoreactivity in these areas (Fig. 4). Stromal cells with CD18 immunoreactivity were occasionally found in the cornea of less affected and unaffected dogs. No GAG accumulation was noted in the corneal epithelium or endothelium on light microscopy (hematoxylin and eosin, Alcian blue) or ultrastructural examination. Histologic GAG accumulation was occasionally noted in scleral fibroblasts but was not detectable in ciliary epithelium, choroid, retina, retinal pigmented epithelium, or optic nerve. Vacuolation of the corneal endothelium, cells of the iridocorneal angle, and less commonly the ciliary epithelium were noted in both affected and unaffected control animals; these vacuoles did not stain with Alcian blue.

None of the dogs had histologic evidence of retinal or optic nerve atrophy, corneal erosions or ulcers, or significant inflammatory cell infiltrates anywhere in the globe. Twelve of 55 (22%) affected dogs for which the iris was available for evaluation had mild pre-iridal fibrovascular membranes (PIFM) that did not involve the iridocorneal angle. This included four of eight (50%) affected but untreated dogs, eight of 33 (24%) low-dose dogs, and two of 14 (14%) high-dose dogs. All dogs with PIFM had histology scores of 3 (n = 6) or 4 (n = 8). None of the dogs with PIFM had been treated since birth; ages ranged from 14 to 33 months; and the length of treatment ranged from 3.25 to 13.75 months.

**Effects of Aging on Corneal Lesions**

There was a significant difference in the ages of affected dogs with corneal lesions graded 0 compared with those with a histologic grade 3 (adjusted P = 0.02), and those with a grade of 0 compared with histologic grade 4 (adjusted P = 0.001). Similarly, there was a significant difference in the ages of dogs with corneal lesions of histologic grade 1 compared with grade 3 (adjusted P = 0.006) or grade 4 (adjusted P < 0.0001). No other comparisons were statistically significant; however, there was a marked degree of correlation between increasing age and increasing histology grade among affected dogs (r = 0.67; P < 0.0001) (Fig. 5).

**Effects of Treatment on Corneal Lesions**

There was a significant difference between the histologic grades of the high and low ERT groups (adjusted P = 0.003) and the high and no treatment groups (adjusted P = 0.01), although histologic grade did not return to normal (unaffected) levels in the high-dose group. There was no statistically significant difference between the histology grade of the low and no ERT group (adjusted P = 0.91). The association of histologic grade with dose group is presented in Table 1. The animals in the low-dose group had been treated for a significantly longer period than those in the high-dose group (median 7.5 months versus 4 months, respectively; P = 0.0001).

When the high- and low-dose groups were further subdivided to reflect those animals that had been treated since birth (<1 month of age), there was no longer a significant difference in the histology score between the high- and low-dose groups (Table 2). The overall median histology grade was significantly lower (P < 0.0007) for dogs receiving ERT from birth than for dogs beginning ERT at an older age (Table 2). Dogs that began treatment at a young age also had a significantly longer length of treatment (P = 0.0001) than dogs that began treatment later in life (Table 2).
When dogs treated with high- or low-dose ERT were evaluated, the histology grade was not correlated with the length of the treatment course ($r = -17; P = 0.24$); however, the sensitivity of this analysis is limited by the small number of dogs with histology grades of 0, 1, and 2. When adjusted for age, treatment since birth, and duration of treatment, dogs in the low-dose group that also received an intrathecal treatment did not have lower histology grades than dogs that did not receive an intrathecal treatment ($P = 0.96$); dogs in the untreated and high-dose affected groups did not receive IT therapy.

When dose group (high versus low), treatment since birth, duration of treatment, and age were considered among affected dogs, only age of the dog at necropsy ($P < 0.008$) and beginning treatment at birth ($P < 0.009$) were found to have a significant effect on corneal histology grades.

**DISCUSSION**

In this study, ocular GAG accumulation occurred in the corneal stromal cells and scleral fibroblasts, as previously reported. In severe cases, corneal stromal cells are significantly distended by GAG accumulation, and in some cases this progresses to cleft formation; both clearly result in sufficient alterations in collagen arrangement to cause corneal opacity. The alterations in corneal stromal GAG turnover and subsequent Na$^{+}$/K$^{+}$ pump adjustments would also likely contribute to corneal opacity. In most dogs in this study, corneal stromal GAG accumulations were associated with corneal vascularization. Cleft formation and/or areas of stromal necrosis may explain some of the focally dense opacities noted clinically in some dogs (Fig. 1).

In most previous canine reports, only the corneas were examined microscopically, but in one study, evaluation of the entire globe found vacuolation of cells in the bulbar conjunctiva, iris, choroid and sclera; this vacuolation was attributed to GAG accumulation. In the present study, GAG accumulation was not identified in these tissues. In humans with MPS I, ocular GAG accumulation occurs in anterior chamber structures and within the uveoscleral outflow tract, resulting in narrowing of the iridocorneal angle, obstruction to aqueous drainage, and subsequent glaucoma. Additionally, GAG accumulation in the dura mater or sclera can cause compression and atrophy of the optic nerve. GAGs have also been shown to accumulate in retinal ganglion cells or retinal pigment epithelial cells, which may further contribute to optic nerve atrophy and electroretinogram abnormalities. These additional GAG accumulations were not noted in any of the canine globes examined in this study, nor was there any evidence of glaucoma or optic nerve degeneration. This may represent a difference in the manifestations of MPS I in our dogs, which generally exhibited a somewhat smaller form of disease than Hurler patients, or, less likely, an age-related sampling bias. In one human ERT study, ages ranged from 7 to 44 years. In the present study, the oldest affected dogs were 40 and 50 months old, and in both cases only the cornea was available for examination. The only other associated ocular abnormality in the dogs in the present study was the presence of a preiridial fibrovascular membrane (PIFM). Formation of PIFMs is attributed to the effects of soluble vasogenic proteins like VEGF from inflammatory cells, neoplastic cells, or retinal neurons. In dogs, PIFMs most commonly occur in globes with chronic retinal detachment, retinal ischemia, chronic uveitis and intraocular tumors. In severe cases, PIFMs can obscure the iridocorneal angle, impede aqueous outflow, and result in secondary glaucoma. A recent publication describes anterior chamber flare (increased protein in the aqueous) in some untreated dogs with MPS I; this may have contributed to PIFM formation in affected dogs. The PIFMs seen in the dogs in the present study were thin (mild) and did not involve the iridocorneal angle. The presence of a PIFM tended to decrease with increasing treatment dose, but given the small number of affected dogs, statistics were not performed. The underlying cause of PIFM development in these dogs is unclear but may be secondary to corneal pathology; to the best of our knowledge, similar lesions are not reported in humans.

As expected, severity of the GAG accumulation correlated with increasing age of the dog. The dogs whose corneas were a grade 1 were significantly younger than those whose corneas were a grade 3 or 4. CD18 positive, GAG laden cells were increased in the corneas of affected dogs in comparison with the number of CD18 expressing cells in unaffected control dogs. Cell morphology and CD18 expression indicate these

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**Table 1. Median Age at Necropsy, Length of Treatment, and Histologic Grade for All Dogs**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median Age at Necropsy (Range)</th>
<th>Median Length of Treatment (Range)</th>
<th>Median Histologic Grade (Range)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 4)</td>
<td>56.5 (8–97)</td>
<td>NA</td>
<td>0*</td>
</tr>
<tr>
<td>No treatment (n = 9)</td>
<td>19 (3–50)</td>
<td>NA</td>
<td>4 (1–4)b</td>
</tr>
<tr>
<td>Low-dose ERT (n = 35)</td>
<td>19 (13–40)</td>
<td>7.5 (2.25–18)</td>
<td>3 (1–4)b</td>
</tr>
<tr>
<td>High-dose ERT (n = 14)</td>
<td>17 (4.3–30)</td>
<td>4 (0.5–13)</td>
<td>1.5 (0–4)f</td>
</tr>
</tbody>
</table>

* Age at necropsy and length of treatment are reported in months.
* Differences between observations in the same column with different letters are statistically significant (adjusted $P$ value < 0.05).

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**Table 2. Comparison of Histologic Grade in Affected Dogs Treated since Birth (Less Than 1 Month of Age) to All Other Dogs**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median Age at Start of Treatment (Range)</th>
<th>Median Length of Treatment (Range)*</th>
<th>Median Histologic Grade (Range)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-dose ERT, treated since birth (n = 8)</td>
<td>0.6 (0.1–0.7)</td>
<td>17 (15–18)$a$</td>
<td>2 (1–4)$ab$</td>
</tr>
<tr>
<td>Low-dose ERT, all other dogs (n = 27)</td>
<td>15.25 (6–33.75)</td>
<td>6.5 (2.25–13.75)$b$</td>
<td>4 (3–4)$bc$</td>
</tr>
<tr>
<td>High-dose ERT, treated since birth (n = 8)</td>
<td>0.3 (0.1–0.9)</td>
<td>8 (4–13)$b$</td>
<td>1 (0–2)$a$</td>
</tr>
<tr>
<td>High-dose ERT, all other dogs (n = 6)</td>
<td>16.75 (13.75–29.5)</td>
<td>3.25 (0.5–3.25)$c$</td>
<td>3 (3–4)$ad$</td>
</tr>
</tbody>
</table>

* Age and length of treatment are reported in months.
* Differences between observations in the same column with different letters are statistically significant (adjusted $P$ value < 0.05).
are macrophages, which have not previously been reported in dogs or humans with MPS I. Furthermore, to our knowledge, this is the first report of resident stromal macrophages in the normal dog cornea. The presence of macrophages in normal corneas has been reported in mice, guinea pigs, rabbits, and humans. In a mouse model of MPS I, increased numbers of macrophages containing storage material were found in the brain. The increased numbers of macrophages seen in the present study could represent hyperplasia of the preexisting macrophages in response to signals from GAG-laden keratocytes or macrophages. Alternatively, there may be increased recruitment of macrophages from the blood, as the most severely affected dogs had concurrent corneal vascularization. The death of GAG-laden keratocytes is not reported, but it could result in extracellular GAG deposition with subsequent recruitment or proliferation of macrophages.

Dogs in the high-dose ERT group (1.58–2 mg/kg) had significantly less corneal GAG accumulation compared with the untreated and the low-dose treatment dogs. In contrast, there was no significant difference between the low-dose ERT group (0.58 mg/kg) and untreated dogs. In an earlier report, Kakiss et al. found that corneas from dogs treated with 0.5 mg/kg did exhibit enzyme activity, but it was lower than normal. In addition to the IV ERT, 20 of the 35 (57%) low-dose dogs in the present study also received intrathecal treatments, but this additional targeted dose of enzyme had no effect in clearing or preventing corneal stromal GAG accumulation when compared with dogs that did not receive IT enzyme.

**Figure 3.** Transmission electron microscopy of the cornea from an affected dog with grade 4 corneal stromal cell vacuolation. The cytoplasm is expanded by well demarcated vacuoles (lysosomes) containing flocculent granular material (GAG; arrows).

**Figure 4.** Immunohistochemistry for CD18. (A) Cornea of an unaffected control dog. Scattered corneal stromal cells are immunoreactive (brown; arrows); these are interpreted to be resident corneal macrophages. (B) Cornea of an affected dog with grade 3 corneal stromal cell vacuolation. Corneal stromal cells are vacuolated and frequently immunoreactive (brown), demonstrating increased numbers of corneal macrophages.

**Figure 5.** Age of affected dogs compared with histologic grade. *Adjusted P value < 0.01 when age of dogs with a histologic grade of 1 is compared with age of dogs with a histologic grade of 3. †Adjusted P value = <0.0001 when age of dogs with a histologic grade of 1 is compared with age of dogs with a histologic grade of 4.

Dogs that began treatment at birth had significantly less corneal GAG accumulation than those starting treatment at an older age, regardless of the dose used. However, animals that started treatment at birth also had a significantly longer course of treatment, and it is therefore difficult to determine which factor is more important. However, when considering all treatment data together, having a longer course of treatment in itself was not associated with a significantly lower histology score, but starting treatment at a young age was associated with a significantly lower histology score. These findings suggest that starting ERT early can actually prevent some corneal GAG accumulation. The length of the treatment, however, may not be biologically relevant because individuals with MPS require lifelong enzyme replacement therapy. It is also likely that ERT helped clear some previously accumulated GAG in the high-dose group. Unfortunately, the degree of corneal GAG accumulation before the onset of treatment could not be assessed; however, some animals began treatment as early as 2 days of age. A clinical description of the time course and severity of corneal opacity is also not available. The time required for metabolism and excretion of accumulated GAG within the cornea is unknown, but storage material can be reduced in other parts of the body in as little as 2 weeks with five treatments of 0.5 mg/kg (equivalent to the low-dose ERT group in this study). In one human trial, patients who had previously received corneal transplants did not develop corneal opacities after long-term (4-year) ERT. Although corneal transplantation has not been studied in dogs with MPS I, it is likely that it would also be an effective treatment.

The findings in this study suggest that high-dose IV ERT is an effective treatment for corneal stromal GAG accumulation. In addition, starting treatment soon after birth had a significant beneficial effect in preventing corneal storage material; this is consistent with human reports that underline the importance of rapid diagnosis and treatment for individuals with MPS.

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**References**


